Role of Dopamine D3 and Serotonin 2C Receptors in the Development of an Addiction-Like Phenotype in Rats

Valeria A. Acosta, Michelle R. Doyle, Marisa Desai, Gregory T. Collins, PhD
Department of Pharmacology, UT Health San Antonio
About 4.5 million Americans consume illicit substances other than marijuana each month, costing the United States nearly $300 billion per year in healthcare, lost-productivity and crime costs. However, not everyone who consumes these drugs develops Substance Abuse Disorder (SUD). Due to this, our laboratory is interested in assessing individual vulnerability to developing addiction and the underlying mechanisms behind these behaviors. In previous studies, we found that a subset of rats self-administrating 3-4, methylenedioxypyrovalerone (MDPV) exhibit a robust addiction-like phenotype, characterized by notably high rates of drug-intake, schedule-inappropriate responding, and decreased sensitivity to foot shock-punished responding. Dopamine D3 (DA D3) and Serotonin 2C (5H-T2C) receptors have been associated with aberrant drug-taking behavior in rats. The current study aims to assess whether 1) rats that later present with the addiction-like phenotype have increased DA D3 and decreased 5HT-2C receptor function before self-administration, and 2) if these individual differences in drug-taking behavior are exaggerated after rats self-administer cocaine and MDPV. In order to test the above hypotheses, rats were first assessed for individual baseline rates of yawning after receiving injections of increasingly larger doses of either Pramipexole (DA D3 agonist) or Lorcaserin (5-HT2C agonist) at thirty minute intervals to evaluate DA D3 and 5-HT2C receptor mediated behavioral effects, respectively. Then rats self-administered either cocaine (0.32 mg/kg/infusion) or MDPV (0.032 mg/kg/infusion) under a fixed ratio 1 (FR1) schedule of reinforcement until responding was acquired (14 days) and then moved to an FR5 schedule of responding for 10 days. Subsequently, rats underwent a phenotyping period to determine the severity of their addiction-like phenotype by ranking rats based upon 1) drug intake; 2) rates of inappropriate responding; and, 3) sensitivity to foot shock-punished responding. A second assessment of yawning was completed to evaluate changes in DA D3 and 5-HT2C receptor mediated behavioral effects after self-administration. Preliminary results suggest that, prior to any drug exposure, rats that subsequently developed a more severe addiction-like phenotype exhibited a greater response to DA D3 receptor activation, and a diminished response to 5-HT2C receptor activation. These findings suggest that baseline differences in DA D3 and 5-HT2C receptor function exist before any drug exposure, and may be predictive of individual differences in drug-taking behavior. This is important because it suggests a possible mechanism underlying vulnerability to developing a SUD and suggests possible targets for treating SUD.
Type 2 Diabetes Mellitus (T2DM) is characterized by insulin resistance leading to impaired glucose tolerance (i.e. the inability for the body to metabolize glucose) and consequently hyperglycemia. Affecting over 370 million people worldwide, T2DM is tied to both genetic and environmental factors, such as obesity, smoking, inactivity and unhealthy diet choices. There are several existing treatments for T2DM, including Sodium-Glucose Co-Transporter 2 inhibitors (SGLT2i). By inhibiting SGLT2 transporters found in the kidney, these drugs prevent the reabsorption of glucose into the bloodstream, thereby allowing glucose to be excreted in the urine. As a result, it has been shown that SGLT2i can aid in weight loss in patients with T2DM through caloric deficit and increased lipolysis (i.e. fat breakdown). A common rodent model for T2DM is the diet-induced obese (DIO) mice. After just one week on a 60% high-fat diet (HFD), mice gain significantly more weight compared to chow-fed mice. Longer-term HFD feeding leads to overt obesity, ectopic lipid deposition, peripheral and hepatic insulin resistance and glucose intolerance. The aim of this study was to determine 1) whether changes in glucose tolerance in C57BL/6 mice can occur after a short-term HFD (two weeks) and 2) if a short-term (one week) Canagliflozin [10 mg/kg/day] treatment improves glucose tolerance in the HFD mice. We hypothesized that the HFD will negatively affect glucose tolerance and that Canagliflozin will reverse this effect in HFD mice. To address our aim, we performed three intraperitoneal glucose tolerance tests (IPGTT) on C57BL/6 mice (n = 6) using 500 µL of 10% Dextrose following a five-hour fast. Blood glucose was measured at t = 15, 30, 60, and 120 minutes for all mice. After the initial baseline IPGTT, we divided the mice into two groups: one group was kept on the regular chow diet (n = 3) and the other group was maintained on the HFD (n = 3) for two weeks. At the end of the two-week diet, we performed a second IPGTT to assess glucose tolerance between the two groups. On the third week, we gave the mice daily doses of Canagliflozin for one week followed by a final IPGTT to quantitate glucose tolerance in the HFD mice. Our data showed no difference in glucose tolerance between all six mice after the initial IPGTT, as expected. Following the two-week diet, the HFD mice demonstrated elevated fasting and post-prandial glucose levels compared to chow-fed mice. Interestingly, the HFD mice lost weight after only four-days of Canagliflozin treatment. In our ongoing studies, we expect that one-week Canagliflozin treatment will restore glucose tolerance in these mice after the final IP-GTT. These findings demonstrate that even a short-term HFD is sufficient to negatively affect glucose metabolism in mice, which could be reversed with a short-term dosage of Canagliflozin, and can be used to model impaired glucose tolerance.
Tetranectin Accelerates Adipose Tissue Dysfunction Through S6K1 Phosphorylation

Alexander Bekar, George Plasko, Lily Dong, Ph.D.
Department of Cell Systems and Anatomy

Obesity has an established association with insulin resistance, type 2 diabetes mellitus, dyslipidemia, and atherosclerosis, with a heavy burden on the healthcare system. Research has found that aging-related changes in adipose tissue function have a stronger correlation with obesity-related diseases than BMI. Depots of adipose tissue not only function as energy storage, but also in immune modulation, thermogenesis, nutrient sensing, and as an endocrine organ. The decline of these functions drive progression to obesity-related diseases and produce the hallmarks of aging adipose tissue, including adipose redistribution, chronic inflammation, cellular senescence, insulin resistance, lipodystrophy, reduced adipokine production, and reduced beige fat function. Knock out of S6K1, a gene downstream of mTORC1, extends lifespan and reduces the hallmarks of adipose tissue aging without the hyperlipidemia, insulin resistance, and glucose dysregulation of chronic rapamycin treatment. A recent study found that S6K1 co-immunoprecipitates with tagged tetranectin, indicating that tetranectin may function through a direct interaction with S6K1. Immunofluorescence of neural cells after treatment with labeled tetranectin demonstrated tetranectin crossing the membrane into the cytoplasm, resulting in higher S6K1 phosphorylation compared to untreated cells. Our research team identified Tetranectin as a potential adipokine, as DNA microarray data showed it was upregulated after differentiation in both brown adipocyte and 3T3-L1 adipocyte cell lines. Tetranectin, coded by the C-type lectin domain family 3 member B (CLEC3B) gene, was also higher in the serum of obese patients and high fat diet-fed mice, but did not produce a phenotype when knocked out in normal chow-fed mice. To determine the function of tetranectin in vivo, Tetranectin-knockout (TNKO) mice were fed high fat diet, revealing reduced hallmarks of aging, such as adipose redistribution and insulin resistance, similarly to male S6K1 knockout mice. These findings suggest the hypothesis that tetranectin increases adiposity through increased phosphorylation of S6K1. To test this hypothesis, we compared inguinal white adipose tissue (iWAT) from TNKO and wild type (WT) mice using a western blot. In brief, we blotted nitrocellulose membranes, containing the protein from an sds-page gel of homogenized TKNO and WT iWAT, with antibodies against molecules upstream of S6K1 activity. Tetranectin knockout mice showed significantly lower levels of S6K1 phosphorylation, but higher levels of phosphorylation for AKT at serine 308. It has been reported that phosphorylated S6K1 acts negatively on PI3K signaling to form a feedback loop, which may explain the increase in TNKO p-AKT 308. Understanding the mechanism of action for a secreted serum protein Tetranectin may introduce new accessible drug targets to improve the quality of life for patients suffering from obesity-related diseases. Tetranectin may function through a novel pathway regulating adiposity, providing significant understanding and opportunities for treating adipose tissue dysfunction in aging and overnutrition.
Nontoxic hematopoietic stem cell transplantation-based neurotrophic factor therapy for Parkinson's disease

Yuan Yuan Ding, Anton Perera, Anindita Bhattacharjee, Cang Chen, PhD, Guo Ge, MD, PhD, Senlin Li, MD

Department of Medicine, The University of Texas Health San Antonio

Parkinson Disease (PD) is a neurodegenerative disease commonly seen among aged people. The disease is caused by the loss of neurons located in the substantia nigra (SN) of the midbrain. PD patients display symptoms such as resting tremor, rigidity, bradykinesia, and postural imbalance. Many treatments for PD appeared on the market over the years, but current treatments only allow for short-term ease of symptoms without targeting the progress of PD - loss of dopaminergic neurons. The goal for treating PD is using neuroprotective and neurorestorative therapies to heal PD patients with long-term effects.

To reach this goal, glial cell-derived neutrophic factor (GDNF), a potent neuroprotective agent, becomes the desired treatment, especially for targeting nigrostriatal dopamine (DA) neurons. However, delivery of GDNF to the brain has been challenging due to the underlying blood brain barrier (BBB) and poor diffusibility of brain tissue tensions.

Only certain agents are allowed by the BBB to have access to the brain itself. Circulating monocytes/macrophages that originate from bone marrow hematopoietic stem cells (HSCs) is a preferred agent. These cells can access sites of neuronal degeneration and interdigitate with neighboring neurons. HSCs are isolated from donor mice and genetically engineered to express GDNF when they differentiate to macrophages after transplantation in the mouse models (genetic and neurotoxin) of PD. The infiltrated macrophages deliver GDNF to degenerating DA and protect them from further degeneration.

Even though the method to deliver GDNF to neurodegenerative sites using HSCs has been proven to be effective in mice, the prerequisites for transplantation, involving chemotherapy and irradiation, can lead to mortality and morbidity. Therefore, they are not well justified to be used in patients with PD. New HSCT technology have been developed to address it.

During a physiologic steady state, most of the HSCs remain in their specialized niches in the bone marrow. Some might leave their niche and circulate in the blood system. When mobilizers such as G-CSF are administrated, the circulation of HSCs increases tremendously. As the number of HSCs leaving their niche and circulates in the blood is increased, it creates empty niches in the bone marrow. If donor cells are introduced at the peak mobilization period, they compete for niche spaces with endogenous bone marrow cells. Donor cell engraftments reach up to 90% after multiple transplantations.

G-CSF is an FDA approved drug and have been used clinically with limited side effects, but the repeated use of G-CSF in this new HSCT regimen may lead to unexpected adverse effects. My work in the lab, specifying on this concern, used locomotive tests to observe any adverse effects on the mice. The animals were divided into control, G-CSF, and vehicle (saline injected) groups. I observed no significant change in activity levels in the mice, suggesting there are no adverse side effects.

Similar therapeutic strategy can be used to deliver BDNF to the brain to treat Alzheimer’s disease (AD). In my work, using the Y-maze test, I observed impaired memory in 5 x FAD mice
(model of AD) compared with wild-type littermates, lay down a base for further BDNF therapeutic studies.

**Association of Single Nucleotide Polymorphisms with HIV-1 Pathogenesis and AIDS**

Donald Duggan, Gabriella Montemayor, Jacob Avina, Jailene Garcia, Vivek Beeram, Michael Ning, Andrew Carillo, Sunil Ahuja PhD
Department of Medicine, VA Center for Personalized Medicine

The Joint United Nations Programme on HIV/AIDS estimates there were 36.9 million individuals living with HIV in 2017, just under 60% of whom were on treatment. There is no cure for HIV/AIDS; modern Highly Active Anti-Retroviral Therapy (HAART) is the predominant treatment, and just as all others, must be sustained for life, taking a toll both physically and financially on patients. Recent studies have shown that HIV/AIDS pathogenesis is influenced by a variety of host genetic factors, including single-nucleotide polymorphisms (SNPs), multiple of which have been strongly associated with abnormal patient responses to infection. Our lab examines hundreds of SNPs in an attempt to identify ones that have an association with an altered rate of disease progression from initial HIV infection to development of AIDS in patients in order to shed light on potential mechanisms, targets, and markers for new therapies and diagnostics. Candidates are largely sourced from genome-wide association studies (GWAS) that associate thousands of SNPs in a large locus with bodily phenotypes, in this case, immune phenotypes. Our studies are conducted via genotype analysis of the United States Military Natural History Study Cohort, which contains the DNA of over 5000 HIV positive former and current service members. Its high sample number and ethnic diversity, along with its patients’ well documented clinical records and uniform standard of healthcare through the military make it a valuable and reliable group for research. One of the many SNPs that has been studied is rs6886944, on the gene COMMD10. COMMD10 is part of a family of proteins characterized by their highly conserved COMM domain, which is used for protein binding. They have been attributed with many discrete functions, including regulation of copper metabolism, regulation of cell proliferation, participation in ubiquitination pathways, and inhibition and of NF-κB, a factor on which HIV is highly dependant. COMM proteins indirectly inhibit NF-κB by interfering with the ubiquitin pathway that facilitates kB inhibitor protein degradation by the proteasome. rs6886944 is located upstream of COMMD10, and was experimentally associated with decreased counts of CD8+ T-cells, which are highly dependant on NF-κB for differentiation, which likely indicated upregulation of COMMD10. It was hypothesized that rs6886944 would slow disease progression by upregulating COMMD10, which might inhibit HIV transcription like its family members. However, the SNP did not show any significant associations in either the European American ethnic group (HR=0.84, 95%CI=0.783—1.305, P=0.932) or the African American ethnic group (HR=1.04, 95%CI=0.757—1.422, P=0.816). Though the results for this SNP were not positive, roughly one in every hundred SNPs we process turns a positive result. Such a result indicating an association with increased progression speed to AIDS (indicated by an HR greater than 1) could be a therapeutic target or at least a warning marker for increased susceptibility, and a result indicating a decreased rate of progression, or a completely inhibited pathogenesis (indicated by an HR significantly less than 1), could be exploited in the form of a cure, just as with CCR5-Δ32.
Effects of Veterans Team Recovery Integrative Immersion Process (Vet TRIIP) Program on Pain and Stress among Participants

Elizabeth Esparza, Mariluz Gonzalez, Bob Deschner, Dottie Goodsun, and M. Danet Lapiz-Bluhm, PhD, RN, MSCI
School of Nursing, University of Texas Health Science Center at San Antonio
Vet TRIIP

Veterans with history of deployment face several risk factors for long-term mental health problems and reintegration to civilian life including posttraumatic stress disorder (PTSD), depression, chronic pain, mild traumatic brain injury (TBI), and other conditions; which affect their quality of life and their families’ (Lapiz-Bluhm & Peterson, 2014). Evidence-based interventions for PTSD, such as cognitive behavioral therapy, are often associated with high dropout rates and sometimes lack of response. Pharmacological approaches are associated with adverse effects. Complementary and alternative medicine (CAM) approaches have been used as an adjunct treatment for PTSD to engage Veterans on their treatment. Vet TRIIP is a short-term multi-modality complementary integrative immersion program for veterans with PTSD and related symptoms. Vet TRIIP is geared toward the Veteran, the active duty service member, the family members, and caregivers to honor and empower them to create healthy, happy, and productive civilian lives (http://www.vettriip.org/). The services provided through Vet TRIIP are emotional freedom techniques (EFT), aromatherapy, clothes-on therapeutic massage, qigong, Reiki, chiropractic care, meditation, reflexology, and acupuncture in a two-hour session. The participant is offered all services in each session and is at liberty to tailor their session to their preference. The study evaluated the effects of Vet TRIIP on Veterans’ pain and stress.

Veterans attended individual IIP Sessions (N = 8,000; January 2012-June 2018), consisting of Intake, 2-hour Treatment, and Outtake. They completed demographics and stress/pain history (past 4 weeks and current) survey (0-10 scale; 0=none; 10=worst), and participated in 3 activities: aromatherapy, gentle stretching, and EFT. They were escorted to a room to lie on massage tables and receive up to 5 modalities: clothes-on therapeutic massage, energy balancing, chiropractic, and acupuncture/acupressure with relaxing music. After, they were assisted and prepared for Outtake where veteran volunteers assisted with exit surveys (i.e., pain and stress levels, relaxation status, treatment recommendation and rating of modalities). Data were analyzed with significance at p<0.05. Pain and stress levels (past 4-week and current) were not significantly different but was significantly reduced (p<0.05) following IIP; Veterans were relaxed and would recommend the treatment to others. Complementary modalities were all highly rated. Vet TRIIP significantly decreased pain and stress among participants, which may improve their quality of life. This program could potentially support and enhance the engagement of veterans and families/caregivers in counseling services and therapies.
Tau Protein Mediates the Link Between Obesity and Cognitive Impairment in Type 2 Diabetes and Alzheimer’s Disease

Jordan Glassman, Valentina R. Garbarino, PhD, Miranda E. Orr, PhD
Barshop Institute of Longevity and Aging Studies,
University of Texas Health Science Center at San Antonio

Alzheimer’s Disease (AD) is an irreversible chronic neurodegenerative disorder. It is the most common cause of dementia and affects nearly 44 million people worldwide¹. Of the top ten causes of death in the United States, it is the only one with no known cure. AD is histologically characterized by the accumulation of Aβ and tau protein aggregates, as well as neuron loss. Among the risk factors, type 2 diabetes doubles the chances of developing AD, although the link between these two diseases is poorly understood. Previous studies have reported that young (<6-mo-old) tau knockout (tauKO) mice are obese and insulin resistant. Moreover, in early stages of AD pathogenesis, tau protein undergoes loss-of-function modifications, which suggests that tauKO mice may possess an increased risk of developing symptoms of AD with advanced age (e.g., neurodegeneration and cognitive impairment). To test this hypothesis, we studied 20-month-old tauKO and age- and sex-matched wild type (WT) mice on an FVB/C57BL6 F1 genetic background. Additionally, we divided this cohort into control fed and high fat diet (HFD) fed mice in order to further exacerbate obesity and insulin resistance. Twenty-month-old tauKO mice were significantly larger than age- and sex-matched WT mice; female tauKO mice on control diet were 63% heavier than female WT mice on control diet (41.23 grams vs. 25.28 grams, p = 0.0143); male tauKO mice on control diet were 18% heavier than male WT mice on control diet (44.2 grams vs. 37.38 grams, p = 0.0082). Interestingly, however, fasting blood glucose, HbA1C and oral glucose tolerance test (OGTT) were not statistically different between obese tauKO and lean WT mice. Upon HFD feeding, the genotype-dependent differences in body mass were increased in females and maintained in males; female tauKO mice on HFD were 99% heavier than female WT mice on HFD (61.98 grams vs. 31.16 grams, p <0.0001); male tauKO mice on HFD were 15% heavier than male WT mice on HFD (57.94 grams vs. 50.33 grams, p = 0.0074). The OGTT indicated that both genotypes and sexes responded to the HFD as evidenced by significantly higher area under the curve (AUC) compared to control diet fed mice; however, their fasting blood glucose and HbA1C did not differ from control fed mice. Overall these results indicate that twenty-month-old tauKO mice are significantly heavier than WT mice, but display normal glucose handling, and do not become insulin resistant when placed on a HFD.

We performed motor activity and cognitive behavioral tests on tauKO and WT mice on both diets to evaluate the effects of obesity and HFD on behavior. Neither marble burying, a test of repetitive behavior, or nesting, a test of activity in daily living, were significantly different between tauKO and WT mice on either diet. Hind paw temperature sensitivity tests also indicated no difference, denoting intact motor function and peripheral nerve sensitivity across genotypes, sex and diet. However, male and female tauKO mice on both diets displayed significantly lower levels of activity compared to WT mice. This was evidenced in open field analysis (OFA) and novel object recognition (NOR) tasks where all tauKO mice displayed significant decreases in center and periphery entries; rearing behavior; distance; and average and maximum velocity. These differences in total activity in both OFA and NOR trials may be linked to the larger body mass of tauKO mice. Though tauKO mice were less active than WT mice, and spent a significantly increased amount of time in the periphery during OFA as measured by percentage (female tauKO = 85.63% vs. female WT = 75.57%, p = 0.0010; male tauKO = 85.44% vs male WT = 79.63%, p = 0.0305), their NOR results were indicative of normal cognitive behavior. Specifically, female tauKO mice on both diets as well as male tauKO mice on the control displayed equal preference to the novel object compared to WT mice as indicated by percentage of novel object visit time vs. total object visit time. Interestingly, male tauKO mice on the HFD displayed increased preference towards the novel object compared to male WT mice on the HFD (p = 0.0073). This
tauKO behavior is consistent with maintained memory, and the absence of a cognitive deficit. In summary, the increased body size of 20-mo-old tauKO mice is associated with lower motor activity, but not insulin resistance or cognitive impairment. In conclusion our data indicate that in the absence of tau protein, mice maintain a healthy obesity phenotype, which suggests that tau protein is a critical link between type 2 diabetes and AD.

(1) https://www.alzheimers.net/resources/alzheimers-statistics/
Forward, Arts!: Program Evaluation and Benefits among Veteran Participants

Mariluz Gonzalez, Russel Stephenson, Kellen McIntyre, PhD, and M. Danet Lapiz-Bluhm, PhD, RN, MSC
School of Nursing, University of Texas Health Science Center at San Antonio
Bihl Haus Arts

More than 2.6 million veterans have served in the Middle East since 9/11. Each year 7,000 service members transition out of the military at Joint Base San Antonio; more than half decide to stay in Bexar County. Overall, an estimated three in ten have PTSD, traumatic brain injury and/or major depression. More active service men and women have lost their lives from suicide than from combat with reportedly 22 American Veterans ending their own lives each day. Bihl Haus Arts (BHA) launched an innovative program offering professionally taught drawing and painting classes to veterans who are experiencing depression or are clinically or self-diagnosed with Post-Traumatic Stress Disorder (PTSD). The mission of the program, called “Forward, Arts!” is to offer veterans opportunities for healing, wellness and community through engagement in the arts. Forward, Arts! is a drawing and painting class for veterans with PTSD and related symptoms (https://bihlhausarts.org). The services provided through Forward, Arts! are drawing and painting classes over the course of ten weeks. This study aimed to evaluate the effects of the Forward, Arts! Program. Participants (N=8) were asked to complete pre- and post-surveys related to art and their creative process, quality of life, and resilience, and share their feelings when creating art. Data indicate improvement across the measures following program. Themes from the qualitative data about creating art include increased concentration and relaxation, reduction in pain and stress, and improvement of art skills. Some comments from the participants include: “I can concentrate better with more focus—very relaxing.” “It gives me a feeling of peace and joy” “I feel good about what I have created. I feel calm and I am able to put aside all my worries and just focus on my classwork. This class has helped me to take time out for me and just block everything else out.” “I normally have pain and trouble with daily activities but when I am drawing or sculpting, I am in a zone where I forget everything! Stress and pain are very much reduced.” “I am able to relax and let my ideas flow from my mind. I am able to concentrate, which is a big deal. “I have chronic pain also and while I am in the moment of art, I feel no pain.” I feel transformed. I am relaxed. And I am much more focused.” “I’ve always been detail oriented, but this class has enhanced that skill. I feel artsy!” These findings show that Forward Arts! Program was beneficial to the Veteran participants not only in terms of their artistic abilities but more importantly with stress relief and pain control. These data support the continuation of the Program and further dissemination in the community so it can reach the Veteran populations. Veterans are encouraged to participate in the Forward, Arts! program.
In insulin action contains a coordinated set of signals which are essential to homeostasis, including balancing nutrient availability and maintaining metabolism during times of caloric abundance and scarcity. Insulin resistance is the loss of the ability of insulin target tissues to respond adequately to physiological concentrations of insulin, resulting in systematic deregulation of glucose metabolism. Insulin resistance is believed to play a crucial role in the pathogenesis of type 2 diabetes, as well as many other obesity-related diseases. Although it is known that obesity and insulin resistance are interconnected, the mechanism underlying obesity-induced insulin resistance remains uncovered. Protein palmitoylation involves a highly coordinated mechanism which provides the ability to modulate cell signaling. Palmitoylation is a posttranslational process, some of which are reversible by demodifying enzymes known as depalmitoylases. Depalmitoylation is the removal of this lipid group from target proteins, which results in the alteration of the subcellular localization of their target molecules. LF8 has been identified as a potential novel depalmitoylase whose expression positively correlates with adiponectin signaling, insulin sensitivity, and metabolism. In order to test its physiological function, we generated LF8 whole body KO mice. Deletion of the LF8 gene in mice leads to the magnification of the high fat diet-induced obesity phenotype and pronounced hepatic inflammation. The expression of the LF8 gene has been found to be largely restricted to peritoneal macrophages, and its expression triggers macrophage polarization. The results portray a critical role of depalmitoylation in regulating insulin sensitivity and diet-induced chronic inflammation, as well as suggest that the regulation of peritoneal macrophage function contributes to systematic energy homeostasis.
Human immunodeficiency virus 1 (HIV-1) is a virus that destroys the host’s immune system and creates an environment in which the host is susceptible to developing acquired immune deficiency syndrome (AIDS). Within three to four weeks, the host’s CD4+ T-cell counts begin to drop, leading to an inverted CD4+ to CD8+ T-cell ratio. This leaves the host with a weakened immune system susceptible to various opportunistic infections, such as tuberculosis, toxoplasmosis, pneumonia, and more. Various studies have revealed that one genetic mutation, CCR5Δ32, creates a host environment immune to HIV-1 infection. This immunity is facilitated by mutating the CCR5 co-receptor on the CD4+ T-cell, a co-receptor commonly used by HIV-1 as an entryway into the cell. This discovery was a catalyst for host genetic HIV-1 research. In the Personalized Medicine lab, candidate research is conducted on various single nucleotide polymorphisms (SNPs) that have been loosely associated with various immunological traits using DNA from the U.S. Military HIV Natural History Study (NHS) cohort. The NHS cohort was established in 1985 to study HIV, when modern antiretroviral therapy for HIV infected patients was unavailable. Due to the large size, diversity, and long established record of clinical data, the NHS cohort is capable of producing quality data. Quantitative real-time polymerase chain reaction (qPCR) is used to amplify and read the DNA of these HIV-1+ patients. Immune-related SNPs expressed in the host DNA are identified through qPCR; this data is then compared to well-documented clinical data about the host’s onset of HIV infection and progression to AIDS. After statistical analysis is completed, associations between SNP expression and patterns in HIV-1 development are made. SNP rs4074424, located on gene ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1), has been found to be associated with the percentage of CD8+ T-cells expressing ENTPD1. ENTPD1 is implied to have a role in Treg-mediated suppression, a function that could likely influence HIV pathogenesis. For these reasons, rs4074424 is a candidate SNP of interest. Using DNA from the NHS cohort, qPCR, and statistical analysis, the role of rs4074424 in HIV-1 pathogenesis was able to be identified. There was no association with HIV-1 for rs4074424 in this study, but it should be noted that in both ethnic populations studied, African American and European American, the hazard ratios predicted an increased likelihood of the occurrence of an AIDS93 event for heterozygous and homozygous mutant genotypes. However, none of the P values indicated statistical significance for the SNP’s potential associations. The lack of differentiation in allelic distribution between progressors and those who did not progress to AIDS for rs4074424 also does not support an association. However, despite the lack of an association between rs4074424 and HIV-1, these results still identify the SNP’s previously unknown role in HIV-1 pathogenesis.
Palmatine Suppresses Glutamine Induced Proliferation in Pancreatic Cancer Cells through Inhibition of STAT3 Signaling

Varsha Mulamreddy, Xiaoyu Yang, Amanda Muñoz, Divya Chakravarthy, Pratap Kumar, PhD, Department of Urology and Molecular Medicine

Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal malignancy that is largely resistant to chemotherapy and radiation therapy with a 5-year survival rate of less than 8%. Even though recently there have been advancements in treatments for pancreatic cancer, the survival rate is still poor. This is in part due to a phenomenon known as desmoplasia. Desmoplasia is unique to PDAC and contributes to the resistance to conventional therapeutics. This dense desmoplastic response, initiated by the activation of pancreatic stellate cells (PSCs), significantly reduces drug efficiency by providing protection around the tumor with fibrous tissue. The communication between PSCs and pancreatic cancer cells (PCCs) supports the growth of PCCs. Previous studies in Dr. Kumar’s lab found that PSCs secrete significantly higher amounts of glutamine under nutrient-deprived conditions. Notably, the communication between PSCs and PCCs can be inhibited by a natural compound known as Palmatine (PMT). Our data in multiple pancreatic cancer cell lines showed that glutamine stimulation induced increased proliferation of PCCs, which can be suppressed by PMT treatment, however, the mechanism of which is unclear. Our preclinical data showed that the treatment of PMT is associated with significantly lower levels of serum IL-6 - a well-known activator of STAT3 signaling. Since constitutive activation of STAT3 has been reported in pancreatic cancer patients and that STAT3 signaling have been demonstrated to contribute to therapeutic resistance, we set out to study the effect of PMT and glutamine on STAT3 signaling in human pancreatic cancer cell lines. We used two different human pancreatic cancer cell lines namely MIA PaCa-2 and PANC-1 in my studies. The initial studies using immunoblot analysis revealed that glutamine increases levels of pSTAT3 with no significant impact on total STAT3 at 6 h and 24 h. In addition, treatment with PMT also decreased phosphorylated but not total levels of STAT3 in these cells. This data implicates PMT as a potential STAT3 inhibitor. After establishing PMT as a potential STAT3 inhibitor, we tested if PMT could inhibit glutamine induced increased levels of pSTAT3. Our results show that glutamine induced increased levels of pSTAT3 but not total STAT3 can be suppressed with PMT treatment. Biologically, treatment with PMT reduced proliferative ability of these cells. Taken together, these observations suggest that PMT suppresses glutamine-induced proliferation in part through inhibiting STAT3 signaling. Given that STAT3 contributes to therapeutic resistance and that PMT suppresses pSTAT3 and inhibits proliferation of both cancer and stellate cells, we speculate that PMT could potentially reduce therapeutic resistance. Additional studies are required to test this hypothesis.
Monocyte Chemoattractant Protein-1 (MCP-1) is a chemokine that selectively recruits monocytes, lymphocytes, and macrophages. MCP-1 is known to be a stress protein and is upregulated when the cell undergoes any type of extracellular aggravation. When the fibroblasts are treated with doxorubicin, the mRNA coding for MCP-1 is upregulated. The assumption being if mRNA production is increased, this would lead to the translation of the MCP-1 protein. Immunofluorescent chemistry (IFC) to assess MCP-1 expression, depicts the control group with less production of MCP-1 compared to the doxorubicin treated cells. In order to investigate quantitatively the results of the IFC qualitative images, a western blot was run to quantify the amount of protein. The western blot showed that the protein was upregulated in cells treated with doxorubicin. P53 is a suppressor gene that halts transcription and induces apoptosis, if there is a possibility of the initiation of cancer. P53 is also a regulator of MCP-1 expression, particularly during times of cellular stress. Mice with p53 knockout tend to be less healthy and smaller in size but may show stunted upregulation of MCP-1 after treatment with doxorubicin. IFC images showed higher staining in the control groups with p53 knockout and no treatment of doxorubicin compared to knockout cells treated with doxorubicin that showed less production of MCP-1; this was quantified via western blot by comparing integrated density of MCP-1 bands on nitrocellulose from different treatment groups and genotypes. These findings suggest that p53 knockout cells are more resistant to doxorubicin-induced upregulation of MCP-1 protein expression.
Use of Granulocyte Colony-Stimulating Factor in a Novel Hematopoietic Stem Cell Transplant Regimen for Treatment of Parkinson’s Disease

Anton Perera, Yuan Yuan Ding, Anindita Bhattacharjee, Cang Chen, PhD, Senlin Li, MD
Department of Medicine, The University of Texas Health San Antonio

The prevalence of Parkinson’s Disease (PD) and its terminal course have underscored a striking gap in existing methods of treatment. PD causes loss of dopamine producing neurons located in the substantia nigra of the midbrain, resulting in resting tremor, rigidity, bradykinesia, and postural imbalance. Current treatment options only ease the symptoms without modifying the progress of the disease or slowing down ongoing loss of dopaminergic neurons. Neuroprotective and neurorestorative therapies are the long-sought but elusive goal for PD and other neurodegenerative diseases. Glial cell-derived neurotrophic factor (GDNF) is a potent neuroprotective agent, especially for nigrostriatal dopamine neurons. Since underlying causes for the neuronal loss in PD remain uncertain, therapeutic use of GDNF to protect neurons is particularly attractive. However, clinical trials of GDNF in PD by surgery-aided direct brain delivery have proven ineffective. Due to the blood brain barrier (BBB) and the poor diffusibility of brain tissue GDNF administered directly to the brain cannot reach cells outside of the injection site and therefore fails to prevent neuron loss. Circulating monocytes/macrophages, originated from bone marrow hematopoietic stem cells (HSCs), preferentially migrate through the BBB to sites of neuronal degeneration, where they interdigitate with neighboring neurons via their cell processes. Therefore, if the HSCs are genetically engineered to produce GDNF upon being differentiated into macrophages, their transplantation would allow effective application of GDNF to sites of neural degeneration as the macrophages naturally migrate there. This treatment effectively protects against neuronal loss, resulting in amelioration of parkinsonian symptoms in multiple studies. Engineering the HSCs requires harvesting them from the patient and then, after modification, autologously transplanting them back into the patient. Conventional methods of HSC transplant are fairly toxic, involving chemotherapy and/or irradiation, resulting in considerable morbidity and mortality, so they may not be justified for use in patients with non-life-threatening conditions such as PD. To solve this problem, new nontoxic HSC transplant technology has been conceptualized and developed. At physiologic steady state, the majority of HSCs remain in specialized niches in the bone marrow. Nonetheless, some leave their niches and travel into the blood. Egress of HSCs is dramatically increased by administration of mobilizers such as G-CSF, either alone or in combination with additional pharmacological agents such as AMD3100 (Plerixafor). This leads to an increased population of HSCs circulating in the peripheral blood while creating empty niches in the bone marrow. Donor cells introduced at peak mobilization compete for niche space with egressed endogenous bone marrow cells. Donor cell engraftments, measured in terms of donor (GFP+) chimerism in peripheral blood samples of recipient mice, reach up to 90% after multiple transplantations. While the development of this form of transplantation was to implement this lab’s treatment for PD, HSC transplantation has great potential application to a variety of noncritical debilitating diseases including autoimmune diseases, so it is vital to verify that neither drug used will cause toxic side effects.

Both G-CSF and AMD3100 are FDA approved drugs and have been in clinical applications for decades with very limited side effects. However, repeated administration of G-CSF and AMD3100 in large doses in this new HSC transplant regimen may cause unanticipated adverse effects. Therefore, a study was conducted by comparing mice given G-CSF in amounts in line with transplantation requirements, mice given saline, and un.injected control mice to see any indication of the manifestation of adverse effects over a long period of time. We found no significant difference among these groups as evaluated by behavioral tests. Our findings suggest both G-CSF and AMD3100 can be used safely in this new stem cell transplantation method.
Physiological effects of “bath salts” constituents 3,4-methylenedioxyxpyrovalerone (MDPV), methylone, and caffeine in male rats

Riley S. Pritchett, Robert W. Seaman, Gregory T. Collins, Ph.D.
Department of Pharmacology, UT Health San Antonio

In recent years, there has been a substantial rise in the use of illicit designer drugs known as “bath salts” along with an increase in emergency room visits due to medical complications caused by the cardiovascular stimulatory effects of this new class of drugs. Methylone, MDPV, and caffeine are some of the most common constituents found in the preparations of “bath salts.” Methylone and MDPV are synthetic derivatives of the naturally occurring stimulant cathinone and are often found in various drug mixtures containing other stimulants such as caffeine. Preliminary studies suggest that high dose abuse of these synthetic cathinones can lead to lethal cardiovascular events. The aim of the current study is to investigate and characterize the physiological effects of the “bath salts” constituents MDPV, methylone, and caffeine.

Telemetry transmitter devices were surgically implanted in male Sprague-Dawley rats to measure heart rate, mean arterial pressure, core body temperature, and locomotor activity. Rats were habituated to the test chamber for 1-h before receiving an intravenous infusion of either MDPV (0.032-3.2 mg/kg), methylone (0.1-10 mg/kg), or caffeine (0.32-32 mg/kg). Recordings continued for 6 hours following drug administration. Findings for this study thus far show that none of the three drugs significantly affected core body temperature. MDPV, methylone, and caffeine produced an increase in heart rate in a dose-dependent manner with the greatest increase being from methylone. MDPV and caffeine exhibited a similar dose dependent increase in blood pressure, while methylone induced a decrease. Lastly, preliminary data has shown that the administration of all three drugs stimulates locomotor activity. These data show that intravenous administration of either MDPV, methylone, or caffeine alters heart rate, blood pressure, and locomotor activity in a dose-dependent manner while having little effect on core body temperature. Though these data provide evidence regarding the physiological toxicity of synthetic cathinones, future studies will determine the degree to which these effects are enhanced when administered as “bath salts” mixtures.
Granulocyte-Colony Stimulating Factor has Therapeutic Potential for Autism Behaviors


With many promising pre-clinical solutions falling short in clinical trials, the need for a more effective treatment for autism spectrum disorders (ASD) remains critical. In approximately 20% of ASD cases, there is evidence of elevated peripheral serotonin (5-HT) transporter (SERT) function. Yet, in tomography studies, SERT density in brain regions thought to regulate social behaviors is lower in ASD patients than in age-matched volunteers without autism. In addition, there is evidence of altered immune response and inflammation in many patients with autism. Therefore, new treatment strategies may be found through modulation of immune responses. Since pro-inflammatory interleukins (IL) are cytokines that impact SERT (for example, IL-1β enhances function, IL-6 suppresses expression), we investigated granulocyte-colony stimulating factor (G-CSF) as a potential therapeutic for ASD in the black and tan brachury T "Itpr3"/ J (BTBR) mouse model. G-CSF is an endogenous cytokine with neurotrophic, anti-inflammatory, and anti-apoptotic functions in the central nervous system. This was only discovered since its introduction to chemotherapy in the form of filgrastim. Due to these functions, G-CSF is emerging as a potential treatment to protect or restore memory and cognitive flexibility for patients with Alzheimer’s disease and neuromuscular disorders. BTBR mice are a generally accepted mouse model for studying ASD due to their impaired social behavior and enhanced restricted repetitive behaviors. Paralleling patients with autism, BTBR mice have reduced hippocampal SERT density as compared to C57BL/6 (black 6) mice. SERT clears serotonin (5-HT), thereby maintaining neuronal sensitivity for 5-HT which is important for promoting social behaviors. Since we and other researchers found evidence of increased neuro-inflammatory markers (IL-6) in BTBR mice, we hypothesized that G-CSF may restore SERT density in brain and alleviate autism-like behaviors by suppressing IL-6 expression. The average baseline serum level of IL-6 in BTBR mice, 35 ± 8 pg/ml, is more than double that in black 6 mice. We administered 125 µg/kg/day murine G-CSF for 5 days, which reduced serum IL-6 to black 6 levels. Behavior testing began 3 days after the last injection, starting with social preference (3-chamber test) and marble burying. Social dominance was tested 2 days later, and water T-maze testing if cognitive flexibility began 3 days after that. The data gathered was analyzed using a priori power analysis, and was collected by treatment blind observers to ensure rigor and reproducibility of the data. Our data indicated no alterations in social interaction or novelty, marble burying, or dominance relative to the saline controls. This may have been due to timing between injection of G-CSF and testing being too short. However, BTBR mice treated with G-CSF had improved cognitive flexibility, taking less time for reversal learning than saline treated controls. This improved cognitive flexibility came 2 to 3 weeks after treatment, when G-CSF levels and IL-6 levels returned to baseline. We investigated SERT binding sites in the brains of tested mice using [125I] RTI-55 with dopamine transporters blocked. G-CSF treated mice had increased SERT density only in the hippocampus. Our next studies will uncover the timing of G-CSF administration in relation to IL-6 levels, social behaviors and SERT density changes.
The Impacts and Mechanisms of Canagliflozin treatment in Mitochondrial Metabolism

Giovanna Romero, Itzel Flores, Luke Norton, PhD
Division of Diabetes

Type 2 diabetes mellitus (T2DM) is the inability of tissues to utilize glucose due to cellular insulin resistance. Type 2 diabetes is closely associated with obesity and affects approximately 9.4% of the U.S. population. Patients with type 2 diabetes have an increased risk for developing cardiovascular disease. Sodium-glucose cotransporter inhibitors (SGLT2i) alleviate hyperglycemia in patients with T2DM through the urinary excretion of excess glucose, and have recently been shown to significantly reduce cardiovascular death in patients with T2DM. However, the mechanism behind the cardioprotective effects of sodium-glucose cotransporter inhibitors, as well as their potential long-term side effects are unknown. To test this, we treated 14-week-old mice on a high-fat diet (HFD) with canagliflozin, a relatively new FDA approved sodium-glucose cotransporter 2 inhibitor, and observed the impact on mitochondrial function after one week of treatment. Glucose tolerance tests were performed before and after treatment and revealed that the high-fat diet led to insulin resistance and glucose intolerance, which are indicators of type 2 diabetes. Canagliflozin increased the amount of glucose excreted in urine and improved glucose tolerance.

Mitochondria produce ATP from glucose via aerobic respiration quantified by cellular oxygen consumption. Seahorse Bioscience XF24 was used to determine the oxygen consumption rates of mitochondria from skeletal muscle, liver, kidney, and heart tissue. The mitochondria were isolated and provided either pyruvate/malate or palmitoyl carnitine/malate in the absence or presence of ADP to test basal, state III mitochondrial respiration, and overall mitochondrial function. Basal respiration demonstrated the energetic demand of the mitochondria in the presence of substrate without ADP and state III respiration was ADP-stimulated respiration. Isolated mitochondria from the heart of HFD mice displayed increased lipid utilization, which was significantly reduced after short-term canagliflozin treatment. These data suggests that SGLT2i may improve cardiovascular health through targeting mitochondrial metabolism. Currently, further studies are testing the long-term effects of canagliflozin treatment on cardiovascular mitochondrial function.
Cyclosporine A as a Treatment for Social Deficits in Autism

Beril Saygin, Livia Ferreira, Nikhita Pathapati, Brandon Rice, Connie Zhang, Georgianna Gould, PhD
Department of Cellular and Integrative Physiology

Autism spectrum disorder (ASD) is characterized by two core symptoms of social and cognitive deficits and restricted repetitive behaviors, with a wide range of symptom severity. Autism can be modeled in the inbred BTBR mouse strain, which exhibit hallmarks of autism including social and cognitive deficits, and a hyperactive immune system. Cyclosporine A (CsA) is an immunosuppressive drug typically used to prevent organ transplant rejection, and treat rheumatoid arthritis and psoriasis. Because CsA readily crosses the blood brain barrier, it is being investigated for is neuroprotective effect and ability to reduce neural damage, such as in traumatic brain injury. For autism, however, CsA may target immune dysregulation to restore typical levels of serotonin and dopamine expression. CsA has been shown to suppress inflammation and decrease the levels of pro-inflammatory cytokines in pregnant rats. In a previous study, it was found that G-CSF reduces IL-6, a pro-inflammatory interleukin, in BTBR mice. Thus, it was hypothesized that CsA may function in a similar manner to increase sociability in the BTBR mouse model of autism. In this study, CsA (15 mg/kg) was solubilized in olive oil and injected to adult BTBR mice and C57BL/6J (black 6) control mice subcutaneously for four consecutive days. Five days after the last injection, the mice were subjected to a three chamber test and marble burying test, which are behavioral assays assessing sociability and restricted repetitive behaviors, respectively. In the three chamber test, the treatment had no effect on preference for interaction with the stranger mouse, empty cup, or the new stranger. Additionally, CsA had no effect on restricted repetitive behaviors in the marble burying test. Consequently, it seems CsA neither enhances nor diminishes sociability in both the control (C57BL/6J) and autism (BTBR) mice.

Because ASD symptoms persist life long and can vary widely amongst patients, it is crucial to find a variety of treatments that can target some of the symptoms of autism. With so many treatment avenues being explored, finding treatments that may not work is as important as finding ones that do. Additionally, some treatments that show promise in animal models are not as successful clinically. Thus, extensive preliminary studies eliminating treatments that do not show promise are valuable. Although some existing literature suggested that CsA may have a detrimental effect on sociability and behavior, this may not be the case. Thus, further research is required to determine if and how CsA and other potential treatments, such as G-CSF, may target symptoms of autism.
It has been well defined that invariant natural killer T cells (iNKT) are an alternate source of help for B cells and play an important role in tumor immunity. This immunity can be enhanced with the help of biodegradable vaccine carriers. By using nanoparticle (NP) technology, we established that poly-lactic-co-glycolic acid (PLGA) nanoparticles can be loaded with the iNKT cell-activating glycolipid, alpha-galactosylceramide (αGalCer), and tumor-associated antigen. Flow cytometry confirmed that NPs loaded with αGalCer activate iNKT cells to produce IFNα. Initial studies used ovalbumin (OVA)-expressing B16-OVA murine cells as a model of melanoma. We found that prophylactic vaccination of mice with NPs loaded with OVA+ αGalCer increased survival and decreased B16-OVA tumor growth rate. In future studies the naturally expressed melanocyte protein, gp100, will be tested as a next generation target antigen using the same tumor model. The effectiveness of this proof-of-principal vaccine platform demonstrates the potential of harnessing iNKT cells for human anti-tumor therapies.